Biex

PN 9005 96 TESTS
PN 9001 480 TESTS

SalEst™
Store at 2° to 8° C
For *In Vitro* Diagnostic Use

INTENDED USE

The SalEst™ test is intended to detect and measure by enzyme-linked immunoabsorbant assay (ELISA) technology the level of salivary estriol in pregnant women. The device is indicated for use as an aid in identifying risk of spontaneous preterm labor and delivery in singleton pregnancies. The device can be used every 1 to 2 weeks from gestational ages 22 to 36 weeks. The test should be used as a component of the clinician's assessment of risk for preterm labor and delivery.

SUMMARY AND EXPLANATION

A surge in salivary estriol occurs in pregnant women several weeks prior to the onset of idiopathic preterm labor.[1,2,3] Detection of the estriol surge between weeks 22 and 37 of gestation identifies women who are at risk for preterm labor. Early identification of women at risk for idiopathic preterm labor allows for early intervention with tocolytic agents or other therapeutic treatments.

The Biex SalEst™ test is used to monitor salivary estriol levels in pregnant women to help identify those at risk for idiopathic preterm labor. Saliva samples are collected weekly to bi-weekly, from weeks 22 up to 36 of gestation, to detect the presence of an estriol surge. A salivary estriol level equal to or greater than 2.1 ng/ml, in singleton pregnancy, indicates that the patient is at risk for spontaneous preterm labor.

Birth before 37 weeks gestation (preterm birth) occurs in approximately 10% of pregnant women in the United States. Preterm birth is directly responsible for at least three-quarters of the total perinatal morbidity and mortality.[4] Among cases of premature delivery, preterm premature rupture of the membranes (pPROM) precedes approximately one third of preterm births, another third are medically indicated as a result of either maternal complications or fetal decompensation and the remaining third of premature births follows the spontaneous onset of preterm labor.[5]

Attempts to reduce the incidence of preterm birth have focused primarily on identifying women at risk for preterm labor based on their obstetric history and on the early recognition of signs and symptoms of labor.[6] Traditional risk assessment frequently utilizes the scoring system developed by Creasy. According to Danforth's Obstetrics and Gynecology, the Creasy Score identifies 21% of women as high-risk, although this group produces only 25% of all preterm births.[7] According to the ACOG Technical Bulletin[7], 50% of all preterm births occur in the low-risk population. A tool for better identifying the risk of preterm labor among low-risk women does not currently exist.

A variety of tocolytic drugs may effectively halt labor or delay delivery if they are used early in the physiologic process that leads to labor. However, once preterm labor has begun, it can be difficult to arrest the progress contractions and cervical dilation. Additionally, women often have difficulty recognizing the early signs and symptoms of preterm labor. As a result, despite efforts aimed at early detection and therapeutic intervention, a significant reduction in the incidence of preterm birth has not been achieved.[6]

The accurate identification of women at risk for preterm labor, especially those who are designated as low-risk would represent an important advance in prenatal screening. Since at least half of preterm births occur in the low-risk population, improved risk assessment methods for low-risk women could exert a significant impact on infant mortality and morbidity.[6]

Estrogens play an important role in the development and maturation of the placenta and uterus during pregnancy.[10,11] Estriol, one of three estrogens normally found in women is produced by the feto-placental unit during pregnancy.[11] After passing through the placenta into the maternal circulation, estriol can be found in either conjugated or unconjugated forms.[12] Both of these forms exist either bound or unbound to plasma protein. The concentration of salivary estriol corresponds to the level of unbound, unconjugated estriol found in plasma.[13,14,15]

Plasma estriol levels increases steadily throughout pregnancy, reaching their peak just prior to delivery.[16] An increase in the salivary estriol/progesterone ratio occurs prior to the onset of spontaneous delivery both at term and preterm.[17,18] Recent studies also have demonstrated a surge in salivary estriol levels several weeks prior the onset of spontaneous preterm labor, the detection of which can help in the identification of women at risk for spontaneous preterm labor and delivery.[1,2,3] Use of the SalEst™ test as an aid in risk assessment for preterm labor in women with singleton pregnancies will lead to more effective patient management.

PRINCIPLE OF THE PROCEDURE

The Biex SalEst™ test is a competitive polyclonal antibody microplate enzyme immunoassay for in vitro diagnostic use in the quantitative detection of estriol in saliva samples obtained from women after 22 weeks of pregnancy.

In the assay procedure, a specimen obtained with the Biex SalEst™ Saliva Collection kit is subsequently processed by the immunoassay. Estriol in the sample competes for binding sites on polyclonal rabbit antiestriol that has been coated onto the surface of microplate wells. Since the number of binding sites on each well is limited sites occupied by estriol obtained from the sample are not available for binding by the estriol enzyme conjugate.

Prior to adding Calibrators, controls or patient saliva samples a Pretreatment Buffer is added to each well to prepare the well for the raw saliva sample. After pretreatment of the microplate wells, Calibrator solutions that contain increasing amounts of estriol, control samples and patient saliva samples are added to the appropriate antibody-coated wells of the microplate.

Horseradish peroxidase (HRP) conjugated to estriol is added to each well, mixed and incubated for 1 hour. Unbound conjugate is removed by a wash step. After washing, HRP substrate is added to each well and incubated for 20 minutes to allow the development of the color reaction, which is indicative of the concentration of estriol in each patient sample. The reaction is stopped with dilute hydrochloric acid. Color intensity is then read on a microplate reader. The concentration of estriol in the samples is determined using a calibration curve.

MATERIALS PROVIDED

Kit	Product Description	Quantity/ Volume
9001	SalEst™ Salivary Enzyme Immunoassay For The Measurement Of Estriol In Saliva	480 tests
	Anti-Estriol Microwell Strips Coated with rabbit anti-Estriol antibody, bovine serum albumin and polysaccharide stabilizers	Sixty 8-well strips (Contained in five 96 well frames in separate pouches)
	Enzyme-Estriol Conjugate Estriol conjugated to horseradish peroxidase in a phosphate buffered saline solution containing bovine serum albumin, stabilizers and antimicrobial preservative, 0.01 % thimerosal	50 ml
	Substrate Hydrogen peroxide/tetramethylbenzidine (TMBlue®).	50 ml
	Stop Solution 0.9% Hydrochloric Acid - Caution Corrosive	50 ml
	Pretreatment Buffer Phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal	14 ml
	Wash Buffer Concentrate (25x) BIS-TRIS buffered saline with stabilizers and preservative	100 ml
	Estriol Calibrator A, 0.0 ng/ml Phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal	4 ml
	Estriol Calibrator B, 1.5 ng/ml * Phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal (* nominal value actual estriol concentration on label)	4 ml
	Estriol Calibrator C, 3.0 ng/ml * Phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal (* nominal value actual estriol concentration on label)	4 ml
	Estriol Calibrator D, 5.0 ng/ml * Phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal (* nominal value actual estriol concentration on label)	4 ml
	Estriol Calibrator E, 10 ng/ml * Phosphate buffered saline with stabilizers, non-ionic detergent,	4 ml

No.	Product Description	Quantity/ Volume
9005	SalEst™ Salivary Enzyme Immunoassay For The Measurement Of Estriol In Saliva	96 tests
	Anti-Estriol Microwell Strips Coated with rabbit anti-Estriol antibody. bovine serum albumin and polysaccharide stabilizers	Twelve 8-well strips (Contained in one 96 well frame in a sealed pouch)
	Enzyme-Estriol Conjugate Estriol conjugated to horseradish peroxidase in a phosphate buffered saline solution containing bovine serum albumin, stabilizers and antimicrobial preservative, 0.01 % thimerosal	10 ml
	Substrate Hydrogen peroxide/tetramethylbenzidine (TMBlue®).	10 ml
	Stop Solution 0.9% Hydrochloric Acid - Caution Corrosive	10 ml
	Pretreatment Buffer phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal	4 ml
	Wash Buffer Concentrate (25x) BIS-TRIS buffered saline with stabilizers and preservative	20 ml
	Estriol Calibrator A, 0.0 ng/ml Phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal (* nominal value actual estriol concentration on label)	1 ml
	Estriol Calibrator B, 1.5 ng/ml * phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal (* nominal value actual estriol concentration on label)	1 ml
	Estriol Calibrator C, 3.0 ng/ml * phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal (* nominal value actual estriol concentration on label)	1 ml
	Estriol Calibrator D, 5.0 ng/ml * phosphate buffered saline with stabilizers, non-ionic detergent, protein, 0.02% thimerosal (* nominal value actual estriol concentration on label)	1 ml
	Estriol Calibrator E, 10 ng/ml * phosphate buffered saline with stabilizers, non-ionic detergent, protein 0.02% thimerosal (* nominal value actual estriol concentration on label)	1 ml

MATERIALS REQUIRED, BUT NOT PROVIDED

Calibrated micropipettes (25 and 100µl) with disposable tips
Disposable transfer pipettes
Microplate mixer or other suitable mixer
Microwell strip or plate reader with capability to read at 450 nm and 620 nm.
SalEst™ Saliva Collection Kit, PN 9004.
Container for diluted wash solution - 2.5 L for 480 test kit (PN 9001) or 500 ml for 96 test kit (PN 9005)
Incubator, 30° C ± 2° C, non-CO^2
Hazardous waste disposal materials
Distilled or deionized water
Control Material - Commercial controls for saliva assays are currently not available. Diluting plasma control material to appropriate levels can produce multi-level controls. Alternatively saliva can be obtained from non-pregnant women and spiked with estriol to appropriate levels.

An example of the first method is given below. Sigma Diagnostics Ligand Control Set, Catalog No. L3527, Lot: 125H6204 is reconstituted per manufacturer's instruction with distilled water. Each level is further diluted with distilled water to achieve a free estriol level consistent with the levels found in saliva. Table 1 shows the results for this lot.

Table 1
Commercial Control Test
Sigma Diagnostics Ligand Control Set (Diluted)

Level	Free Estriol*	Dilution Factor	SalEst™ Measured Estriol
1	2.1	1/8	.75
2	14.5	1/8	2.1
3	33.3	1/8	4.2

* From Sigma Package Insert

INSTRUMENTS

The instrument used in the analysis of results should match or exceed the following characteristics.

Specifications
Wavelength: dual wavelength, 450 nm and 620 nm
Bandwidth: ≤ 12 nm
Absorbance range: 0 absorbance to ≥ 2.5 absorbance units
Reproducibility: ± (0.3% and ± 0.005 AU)
Linearity: ± 0.5% for readings between 0.1-3 AU

WARNINGS AND PRECAUTIONS

Handle all human specimens as if they are potentially infectious.
-Do not pipette by mouth.
-Do not eat, drink, smoke, or apply cosmetics in areas where specimens are handled
-Wear protective clothing such as lab coats and disposable gloves when handling specimens and assay reagents. Wash hands thoroughly afterwards.

Dispose of all specimens and used assay components as biological waste.
Do not contaminate reagents when removing aliquots from reagent bottles. The use of disposable pipette tips is recommended.
Do not use the kit or components after expiration date.
Do not mix reagents or exchange components from SalEst™ Salivary Estriol kits with different lot numbers.
This kit contains no more than 0.01% mercury, wt/vol, as thimerosal. Handle and dispose of properly.

STORAGE CONDITIONS

Store the kit at 2 to 8° C.
Allow kit to reach room temperature (18°-25° C) before using.
Do not expose the kit components to intense light, direct sunlight or temperatures above 30° C or below 2° C

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COLLECTION AND PREPARATION OF SPECIMENS

Collection of Saliva

The patient will collect the saliva sample using the Biex Saliva Collection kit. Saliva is collected weekly or biweekly from week 22 to week 36 of gestation. Complete instructions for the patient are provided with the Biex Saliva Collection Kit.

The Collection Kit is composed of individual self-contained shrink-wrapped collection units. Each collection unit contains a capped saliva collection tube, a plunger with preservatives, a funnel to aid in collecting the saliva. These components are contained within a molded plastic case that serves to provide leakproof mailing of sample collections. Contained within the mailer is an absorbent pad to collect any leakage. Patient instructions and a pre-addressed mailer to return the sample to the laboratory are packaged with the plastic case in a shrink-wrapped box.

The collection tube consists of a 13 mm X 100 mm test tube with a screw-cap closure. The tube has a label the includes an indicator band that serves to provide visual confirmation that sufficient volume, 1 ml, has been provided. The label also contains a bar code that identifies the tube. The collection tube also consists of a plunger device that slides into the test tube after the sample has been provided. The plunger serves the dual purpose of filtering and introducing antimicrobial agents into the sample. After sealing the collection tube with the plunger in place, the filter creates an inner tube sealed on one end by the filter and

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The collection units will also be packaged as single Collection Kits. The single Collection Kit will consist of a collection unit, a patient insert and an enrollment card. The enrollment card will be used by the physician to enroll the women into the screening program, as well as to provide identification and medical history information for the laboratory. The single Collection Kit enrollment form will also include a tracking identification number that has been linked to the collection tube within its respective single-pack kit in a database at the time of assembly.

NOTE: Saliva should be collected between 9 a.m. and 8 p.m.

Preparation of Saliva

No sample pretreatment steps are required prior to analysis. The samples should be collected and stored according to the instructions contained in the Biex SalEst™ Estriol Collection Kit (PN 9003). A sample of the saliva should be drawn from the meniscus to avoid any precipitate that has formed. If it is necessary to assay the saliva between 2 and 24 hours after its collection, the saliva should be vortexed and centrifuged prior to removing a sample to eliminate any precipitate. For accurate quantitation it is important not to pipette any of the precipitate.

PROCEDURE

Reagent Preparation

The microwells, antibody reagent, pretreatment buffer, Calibrators, substrate solution and stop solution are provided ready to use.

1. Wash Buffer Concentrate (25X) - Dilute the wash Buffer Concentrate1:25 with distilled water. To dilute the entire bottle (20 ml), add the contents to 480 ml (2,400 ml for the 480 test kit #9001) of distilled/deionized water. The working dilution should be stored at 2-10° C. The expiration date of the prepared wash buffer is identical to the expiration date of the Wash Buffer Concentrate. Smaller quantities may be prepared by diluting 1 volume of 25X Wash Buffer Concentrate with 24 volumes of distilled/deionized water.

2. Prior to assay, warm all reagents to ambient temperature by allowing them to stand at room temperature or by briefly warming in a 37° C water bath. Gently mix all reagents.

Test Procedure

NOTE: Follow directions exactly, failure to do so may lead to false results.
Perform all assay steps in the order given and without any appreciable delays between the steps.
Three levels of controls should be run with each plate of patient samples.
A maximum of one plate should be set up (completed up to the incubation stage) at a time. If multiple plates are being run as a batch, each plate must be treated as a single entity, i.e. the Calibrators controls and patient specimens for the plate must be added and the incubation time started before moving onto the next plate

Careful attention to pipetting is essential for achieving precise and accurate results.

1. **Prepare assay reagents.** Allow all test components to reach room temperature. Prepare wash solution according to instructions in the Reagent Preparation subsection, above.
2. **Prepare microwell plate.** Determine the appropriate number of microwell strips needed for the patient specimens, Calibrators and controls. See Table 2 below for the recommended positions for the controls and Calibrators. Remove extraneous strips from the plate frame and replace them with null strips (if required). Avoid handling the bottoms of the microwells because scratches or marks could effect the reading of test results. Store unused strips in the original pouch with the desiccant material and seal carefully. Record the microwell position of each patient specimen and control on a laboratory data sheet.

Table 2
Calibrator and Control
Suggested Microwell Position

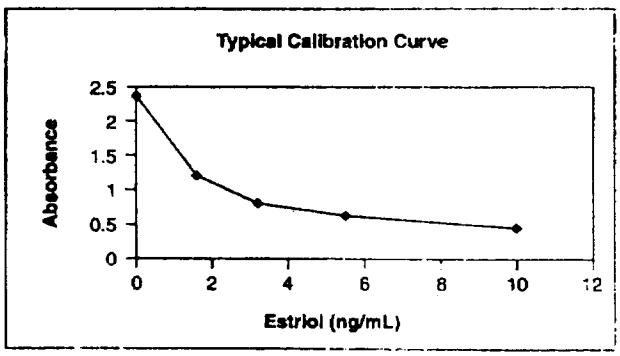
Calibrator/Control	Position
Calibrator A	A1,B1
Calibrator B	C1,D1
Calibrator C	E1,F1
Calibrator D	G1,H1
Calibrator E	A2,B2
Low Control	C2,D2
Mid Control	E3,F3
High Control	A5,B5

1. **Add pretreatment buffer.** Pipette 25 µl of Pretreatment Buffer into the appropriate wells. Allow the plate to rotate on a microplate mixer for 3 minutes.
2. **Add Calibrators.** Pipette 25 µl of Estriol Calibrators in duplicate into the appropriate wells.
3. **Add patient samples.** Pipette 25 µl of patient saliva sample and control(s) into the appropriate wells.
4. **Add Estriol Enzyme Conjugate.** Pipette 100 µl of Enzyme Conjugate into each well. Mix thoroughly for 3 minute on a microplate mixer.
5. **Incubate.** Incubate at 30° C ± 2° C for 60 ± 10 minutes. **DO NOT USE A CO_2 Incubator.**
6. **Wash microwell plate.** Aspirate the liquid from the microwells and wash each well 3 times with approximately 500 µL of wash solution per well. Completely remove all the fluid from the wells after the last rinse.
7. **Add Substrate.** Pipette 100 µL of Substrate into each microwell.
8. **Incubate.** Incubate at room temperature for 20 ± 2 minutes. Avoid exposing the microwells to direct or intense light.
9. **Add stop solution.** Pipette 100 µl of Stop Solution into each microwell.
10. **Read results.** Within one hour after addition of the stop solution, read the absorbance values of the microwells with the microwell reader set at 450/620 nm. Record the absorbance value of each Calibrator, specimen and control.

Evaluation of Results

Calculate the average absorbance value for each Calibrator, control and specimen pair.

Construct a standard curve from the results obtained for the Estriol Calibrators by plotting the average absorbance for each of the Calibrators on the vertical Y axis and the corresponding estriol concentration for that Calibrator on the horizontal X axis.



Draw a smooth curve through the points. Using the calibration curve, interpolate the average absorbance for each saliva estriol specimen and for each control. Record the estriol value(s).

- **Invalid results.** Patient results must not be reported for invalid runs. The assay should be repeated if any of the following conditions apply:

  1. More than one of the controls is unacceptable due to failure to fall within established control limits

  2. One of the Calibrators has a bad duplicate result as defined above

  3. The plot of the calibrator absorbences and estriol values do not result in a monotonically declining smooth curve.

INTERPRETATION OF RESULTS

- Patient specimens with estriol values equal to or greater than 2.1 ng/ml are positive

- Test results should be interpreted in conjunction with the patient's clinical presentation and other diagnostic test results. A negative result by any method does not rule out the possibility of preterm labor and delivery.

- **The physician should interpret positive results with caution. The physician should encourage the patient to submit an additional sample to confirm the initial positive result.**

- ***LIMITATIONS***

The Biex SalEst™ test is used as an aid in identifying pregnant women with singleton pregnancies at risk for spontaneous preterm labor and preterm delivery. The test should not be used alone in pregnant women with suspected rupture of membranes, vaginal infections, uterine anomalies or those who are otherwise already diagnosed as having preterm labor and/or when treatment with betamethasone and tocolytics is being administered. The SalEst™ test should not be used in conjunction with bleeding gums, intrauterine growth retardation, or the presence of fetal demise. The test should be used as a component of the clinician's assessment of risk for preterm labor and delivery.

Specimens measured 3 - 4 hours after collection could have artificially lower values.

EXPECTED VALUES

Listed in Table 3 below are the geometric means by gestational weeks (excluding all patients treated with Betamethasone or tocolytics) of the highest intra-patient estriol value recorded within the intervals used to calculate the average value.

Table 3
E3 Levels (Geometric Means)
By Gestational Age, Weeks

Age, Week	PTL with PTD	No PTL with Term Delivery	P-value
20-<22	0.72 (n=4; 0.45 - 1.15)	0.61 (n=121; 0.42 - 0.89)	0.403
22-<24	0.79 (n=16; 0.51 - 1.20)	0.74 (n=357; 0.51 - 1.06)	0.499
24-<26	0.96 (n=23; 0.74 - 1.24)	0.85 (n=564; 0.61 - 1.18)	0.018
26-<28	1.06 (n=23; 0.81 - 1.37)	0.93 (n=572; 0.67 - 1.30)	0.076
28-<30	1.09 (n=22; 0.86 - 1.78)	1.02 (n=568; 0.73 - 1.42)	0.561
30-<32	1.29 (n=23; 1.07 - 1.55)	1.14 (n=570; 0.81 - 1.61)	0.005
32-<34	1.43 (n=23; 1.05 - 1.94)	1.30 (n=575; 0.94 - 1.80)	0.173
34-<36	2.01 (n=23; 0.94 - 4.29)	1.61 (n=572; 1.07 - 2.42)	0.183
36-<37	2.70 (n=9; 1.76-4.13)	1.77 (n=521; 1.15 - 2.73)	0.004
30-<37	2.30 (n=23; 0.88 - 3.73)	1.72 (n=578; 0.55 - 2.90)	0.009

The relationship of the salivary estriol value and the probability of the onset of preterm labor and delivery are discussed below.

PERFORMANCE CHARACTERISTICS

Analytical Performance Characteristics

Sensitivity

The sensitivity of the test is defined as the lowest concentration of estriol that can be distinguished from the 0 ng/ml Calibrator. The sensitivity of the test is 0.19 ng/ml.

Precision

A precision study was conducted to determine the within-run, between-run and total precision for the kit Calibrators and the controls. Precision was determined with respect to both the optical absorbances and concentrations.

The assay was conducted for 14 days using a fresh kit each day for three kit lots. Standard curves were generated using duplicates of each standard and low-, mid- and high-level estriol controls. Five replicates were run for each test. The data were analyzed for the within-run, between-run and total components of variance that spanned the ODs for all potential sample concentrations and for the concentrations of the controls.
Table 4 presents the results for one of the three lots tested.

Table 4
Precision
Kit LN:0027H6

	Mean OD Conc.	CV, % Within-Run	CV, % Between-Run	CV, % Total
Standard A	2.72	1.1	7.2	7.3
Standard B	1.33	2.6	8.5	8.9
Standard C	0.95	2.8	9.2	9.7
Standard D	0.71	4.0	9.5	10.3
Standard E	0.43	2.4	11.2	11.5
Low Control OD	1.77	3.0	9.0	9.5
Low Control Conc.	1.0 ng/ml	6.4	6.8	9.3
Mid Control OD	1.06	4.0	9.2	10.2
Mid Control Conc.	2.3 ng/ml	9.3	5.0	10.6
High Control OD	0.80	4.8	10.1	11.1
High Control Conc.	4.3 ng/ml	7.3	3.2	8.0

Recovery

This study was designed to determine the accuracy of recovery of the SalEst™ test when a known amount of estriol is added to saliva. Two separate analyses were performed. The first was a linear regression analysis of the standard curves of spiked samples. The second used a single spike level in multiple samples and a t test on multiple replicates to determine if there was significant difference between the observed and expected values.

The following materials were used during this study.

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- Reagents from three lots, 27H6, 28H6, and 29H6
- Negative saliva (*<0.5* eg/ml Estriol)
- 100 ng/ml estriol solution made from a primary reference solution
- 40 clinical specimens from pregnant women obtained between 24 to 35 weeks of gestation.

In the first analysis, standard curves were generated for each sample that was tested. The buffer and saliva were spiked to produce final concentrations of 1,2,4 and 8 ng/ml estriol and the concentrations of the spiked materials was determined using a cubic spit fit to the standard curve. The spiked samples for three lots of reagents were tested in quadruplicate. Buffer results vs. saliva results were plotted and the corresponding slope, y-intercept and correlation coefficient was

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Table 5
Spike Recovery
Linear Regression of Standard Curves

Lot	Slope	Y-Intercept	Correlation Coefficient
27H6	1.1	-0.070	1.000
36115	1.1	-0.167	0.982
29116	1.0	-0.086	0.990

Multiple Sample Recovery

Each of the 40 samples was divided into two aliquots and one set of aliquots was spiked with 2 ng/ml estriol. All samples were tested using the SalEst™ test method described in the package insert. Spiked and unspiked pairs were tested on the same plate and their location on the plate was randomized.

Three values for a given sample were generated by each method: the spiked value, the unspiked value, and the difference between these two values. Spike recovery was assessed using a t-test to determine if the mean differences between all paired spiked and unspiked values were distinguishable from 2.0. Table 6 is a summary of this experiment.

Table 6
Results Summary (N = 40)

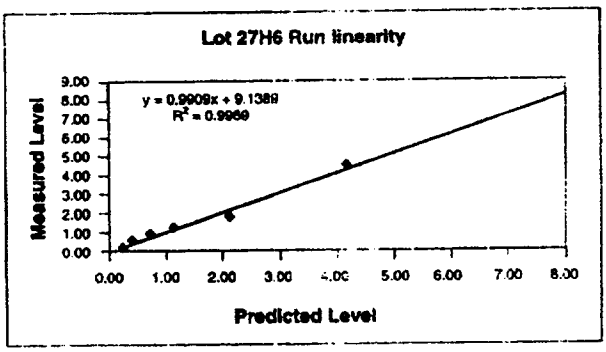
Mean (spiked - unspiked)	Mean distinguishable from 2.0? (p)
1.9	No (0.13)

SUMMARY: This study which examined 40 patient samples, demonstrates that there was 95% (1.9/2.0) recovery.

Linearity

A Saliva Pool containing a known amount of estriol was serially diluted with Calibrator diluent. The observed and expected results are presented below in Figure 1 and demonstrate acceptable linearity after dilution.

Figure 1
Linearity



Specificity

The Biex SalEst™ test is specific for estriol, there is cross-reactivity with estriol-3-sulfate, estriol-3-glucuronide, 16 epiestriol and estriol- 1 6-glucuronide . There is little or no cross-reactivity with other hormones tested. Table 7 contains the list of hormones tested.

Table 7
Cross-Reactivity Results

Compound	Cross Reactivity (%)
estriol-3-glucuronide	52
estriol-3-sulfate	50
estriol-16-glucuronide	6
estrone-3-glucuronide	<1
estrone-3-sulfate	<1
estradiol	<1
17-α-estradiol	<1
17-epiestriol	<1
16-epiestriol	11
cortisone	<1
11-deoxycortisol	<1
5-α-androstenedione	<1
digoxigenin	<1
digoxin	<1
progesterone	<1
pregnanediol glucuronide	<1
testosterone	<1
Estriol	100

Clinical Performance Characteristics

A Pivotal Study was performed on 956 singleton pregnant women to evaluate the ability of the Biex SalEst™ estriol test to identify women at risk for preterm labor. The patient population, which was comprised of 213 evaluable high-risk and 501 evaluable low-risk patients was initially assessed for risk using a modified Creasy score. The primary clinical end point compared the difference in the incidence of PTL resulting in a PTD between patients who had a high E3 level (≥2.1 ng/ml) to those who had a low level (< 2.1 ng/ml) prior to 36 weeks gestation. The data were analyzed using Fishers exact test (2-tailed) with $\alpha = 0.05$. The group of primary interest is women not treated with betamethasone and tocolytics since betamethasone is known to suppress E3 levels and confound test results. The primary group analyzed were PTL and PTD and term delivery (TD) with no PTL. (Patients with pPROM and no labor and medically indicated PTBs were not considered spontaneous PTL.)

The Pivotal Study contained a total of 801 evaluable patients. Provided below are specific performance characteristics of the SalEst™ test and corresponding 2 x 2 contingency tables, Tables 8-10, for the study population, as well as the low- and high-risk subgroups presented separately. For purposes of the primary analysis, the incidence of PTL with PTD in patients who had an estriol ≥ 2.1 ng/ml is compared to the incidence in those patients who had an estriol < 2.1 ng/ml.

A test is considered positive at ≥ 2.1 ng/ml prior to 36 weeks. The analytical sensitivity of the assay is 0.19 ng/ml. The total analytical CV of the system is approximately 10%.

Table 8
Primary Endpoint Contingency Table
Total Study Group (n=601)

	PTD	TD	TOTALS
<2.1	10	449	459
≥2.1	13	129	142
Totals	23	578	601

Performance Characteristics

Fisher's Exact Test: p = <0.001*
Sensitivity = 56.5% (34.5 -76.8)**
Specificity = 77.7% (74.1-81.0)
NPV = 97.8% (96.0-99.0)
PPV = 9.2% (5.0-15.2)
M-H test for combined evidence: p=0.002*
* Significant at the .05 level
** 95% binomial confidence limits are presented in parentheses.

Relative Risk =4.20 (1.88 -9.38)
Incidence +E3 (Incidence of Pos)= 23.6%
Incidence of PTL with PTD=3.8%

Table 9
Primary Endpoint Contingency Table
Low-Risk Patients (n=449)

	PTD	TD	TOTALS
<2.1	6	353	359
≥2.1	6	84	90
Totals	12	437	449

Performance Characteristics

Fisher's Exact Test: p= 0.018 *
Sensitivity = 50.0% (21.1-78.9)**
Specificity = 80.7% (76.7-84.4)
NPV = 98.3% (96.4-99.4)
PPV = 6.7% (2.5-14.0)
* Significant at the .05 level
** 95% binomial confidence limits are presented in parentheses.

Relative Risk = 3.99 (1.32 - 12.08)
Incidence – E3 (Incidence of Positivity) = 20.0%
Incidence of PTL with PTD = 2.7%

Table 10
Primary Endpoint Contingency Table
High-Risk Patients (n=152)

	PTD	TD	TOTALS
<2.1	4	96	100
≥2.1	7	45	52
Totals	11	141	152

Performance Characteristics

Fisher's Exact Test: p=0.047
Sensitivity = 63.6% (30.8-89.1)
Specificity = 68.1% (59.7-75.7)
NPV = 96.0% (90.1-98.9)
PPV = 13.5% (5.6-25.8)

Relative Risk = 3.4 (1.03-10.97)
Incidence+ E3 (Incidence of Positivity) = 34.2%
Incidence PTL with PTD = 7.2%

Table 8 presented above shows that the incidence of spontaneous PTL and PTD is significantly increased in patients with salivary estriol levels ≥ 2.1 ng/ml prior to 36 weeks in the study population (combined high and low-risk women).

The second table, Table 9, indicates that in low-risk women elevated salivary estriol levels identify nearly 50% of the cases of spontaneous preterm labor and delivery that would otherwise have been missed by traditional risk assessment methods. That is, of the 12 cases of PTL with PTD that occurred in the low-risk group as defined by Creasy, SalEst™ identified 6 (Sensitivity = 50%). This results in a 50% increase in detection over traditional risk factors. Thus, the detection rate for PTL and PTD is higher in low-risk women when estriol is ≥2.1 ng/ml than when estriol is < 2.1 ng/ml.

Re-screening Test Results

The use of a re-screen test in women with a single positive estriol test before 36 weeks of gestation was evaluated. The purpose of the re-screen was to determine whether a repeat test enhanced a patient's risk profil relative to a single elevated estriol value. Several factors were analyzed, including the incidence of PTL with PTD and the time to delivery from the second consecutive positive estriol result. In the high-risk subgroup, results of the re-screen were also compared to those of traditional risk factors. The re-screen analysis examined the effects of two consecutive positive SalEst™ test results during the course of serial monitoring of estriol prior to 36 weeks of gestation. Shown below are 2x2 contingency tables, Tables 11-13, and the performance characteristics illustrating the results of these analyses in the total combined population and in the high- and low-risk subgroups.

Table 11
Re-screen
Primary Endpoint Contingency Table
Total Study Group (n=601)

	PTD	TD	TOTALS
<2.1	13	534	547
≥2.1	10	44	54
Totals	23	578	601

Performance Characteristics

Fisher's Exact Test: p=<0.001*
Sensitivity = 43.5% (23.2-65.5)**
Specificity=92.4% (89.9-94.4)
NPV = 97.6% (96.0-98.7)
PPV = 18.5% (9.3-31.4)
M-H Test for combined evidence: P = .000001*
* Significant at the .05 level
** 95% binomial confidence limits are presented in parentheses.

Relative Risk = 7.79 (3.59-16.92)
Incidence + E3 (Incidence of Positivity) = 9.0%
Incidence PTL with PTD = 3.8%

Table 12
Re-screen
Primary Endpoint Contingency Table
Low-Risk Patients (n=449)

	PTD	TD	TOTALS
<2.1	7	407	414
≥2.1	5	30	35
Totals	12	437	449

Performance Characteristics

Fisher's Exact Test: p = 0.001*
Sensitivity = 41.7% (15.2-72.3)**
Specificity = 93.1% (90.5-95.2)

Relative Risk = 8.45 (2.83-25.24)
Incidence + E3 (Incidence of Positivity) = 7.8%
Incidence PTL with PTD = 2.7%

Table 13
Re-Screen
Primary Endpoint Contingency Table
High-Risk Patients (n=152)

	PTD	TD	TOTALS
<2.1	6	127	133
≥2.1	5	14	19
Totals	11	141	152

Performance Characteristics

Fisher's Exact Test: p = 0.005*
Sensitivity = 45.5% (16.8-76.6)
Specificity = 90.1% (83.9-94.5)
NPV = 95.5% (90.4-98.3)
PPV = 26.3% (9.2-51.2)

Relative Risk = 5.83 (1.97-17.27)
Incidence + E3 (Incidence of Positivity) = 12.5%
Incidence PTL with PTD = 7.2%

*Significant at the 0.05 level

As is clearly evident use of a re-screen test following an initial positive result further enhanced results with a P =< 0.001 for the entire study population, p = 0.001 for the low-risk subgroup and p = 0.005 for the high-risk subgroup. Moreover, overall elevated estriol carried a relative risk of 7.79 for the combined group, 8.45 for the low-risk subgroup and 5.83 for the high-risk subgroup. These compared favorably to traditional risk factors such as a twin pregnancy or prior preterm birth which carry a reported relative risk of 2.0 - 2.5. Additionally, less than 10% of the study population was identified as at risk, minimizing the number of false positives.

When the SalEst test is compared to Creasy scoring in the combined high and low risk population, salivary estriol correctly predicts the appropriate outcome 91% of the time, while the Creasy method correctly predicts the appropriate outcome 75% of the time (see Table 14).

TABLE 14

McNemar Test Results Comparing SalEst Rescreen and Creasy

	Creasy Risk Assessment		
SalEst™	Correct	Incorrect	Total
Correct	412	132	544
Incorrect	36	21	57
Totals	448	153	601

McNemar test results: p = <0.001
Odds Ratio = 3.67 (95% CL 2.54 - 5.30)

Time to Delivery

A single positive test and rescreen demonstrated the clinical utility of SalEst test in predicting the probability of delivering within a 5-week time frame, regardless of gestational age. Among women who had preterm labor and delivery and who had a positive SalEst test, the rescreen enhanced the accuracy of predicting delivery within 1 to 5 weeks. In this subpopulation, women who had a positive rescreen had a 63% chance of delivering within 1 week, an 88% chance of delivering within 2 weeks, and a 100% chance of delivering within 3 weeks (see Table 15).

Table 15
Predicting Delivery within 1 to 5 Weeks of a Single Positive Test or a Positive Rescreen
(Patients who had preterm labor and delivery)

No. Weeks To Delivery	Percentage (%) of Patients with estriol ≥ 2.1 ng/ml	
	Single Positive Test n=14 % (CL)	2 Consecutive Positive Tests n=8 % (CL)
<1	28.6% (12.2 - 73.3)	62.5 (24.5 - 91.5)
<2	57.1% (28.9 - 82.3)	87.5 (47.4 - 99.7)
<3	85.7% (57.2 - 98.2)	100 (63.1 - 100.0)
<4	92.9% (66.1 - 99.8)	100 (63.1 - 100.0)
<5	92.9% (66.1 - 99.8)	100 (63.1 - 100.0)

In order to explore whether what occurred in the subpopulation that developed preterm labor and delivery reflected the underlying biology of salivary estriol, the time to delivery of the entire study population that had at least one positive SalEst test result (n=465) was examined (see Table 16). Again, the re-screen enhanced the accuracy of predicting delivery within 1 to 5 weeks. A single positive SalEst test indicates a 78% likelihood of delivering within 5 weeks, compared to a 92% likelihood of delivering within 5 weeks after a positive re-screen Similarly, a positive re-screen indicates a 71% likelihood of delivering within 3 weeks, and an 84% likelihood of delivering within 4 weeks. The time to delivery from elevated estriol is 1-3 weeks in the majority of women.

TABLE 16

Predicting Delivery Within 1 to 5 Weeks
Of a Single Positive Test or a Positive Rescreen
(all evaluable patients)

Weeks To Delivery	Probability of Delivering	
	Single Test (n=465) % (CL)	Rescreen (n=302) % (CL)
<1	15.7%(12.51-19.33)	22.2%(17.63-27.30)
<2	30.5%(26.38-34.95)	50.0%(44.22-55.78)
<3	51.4%(46.75-56.03)	70.9%(65.38-75.92)
<4	68.4%(63.95-72.59)	83.7%(79.12-87.75)
<5	78.3%(74.25-81.94)	92.4%(88.79-95.11)

Length of Time No delivery

The accuracy of the SalEst™ test in predicting no delivery was also examined. The analysis is based on a subgroup of all evaluable patients who met the criteria described above. A negative SalEst™ test result (estriol < 2.1 eg/ml) predicts the likelihood of not delivering within the ensuing 2 weeks (see Table 17). For example, when a sample collected at 26 weeks is negative, the woman has a 99% chance of not delivering within the next week, and a 99% chance of not delivering before week 28. When she collects her next sample, at week 28 if samples are collected biweekly, a new negative sample will project her chances of not delivering before week 30.

TABLE 17

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Predicting No Delivery Within 2 Weeks after a Negative SalEst Test

Sample Collection (Gestational)	Subsequent Two Weeks of Gestation	Likelihood of not Delivering (%)
26 Weeks	27	99
	28	99
28 Weeks	29	99
	30	98
30 Weeks	31	98

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Although Biex, Inc. recommends a salivary estriol value of 2.1 ng/ml or greater be considered a positive result, there may be clinical significance attached to estriol values above or below the 2.1 ng/ml cut-off. Cut-off values below 2.1 ng/ml may identify more true positives (increased sensitivity), but also have more false positives. Cut off values above 2.1 may identify fewer true positives, (decreased sensitivity), but have fewer false positives. A range of values around the 2.1 "cut-off" from ROC curves is given in Table 18 below.

TABLE 18

Predictive Values for SalEst Cut-Off (1.8 ng/ml-2.4 ng/ml)

Salivary Estriol Value	Sensitivity (True Positive)	Specificity	1-Specificity (False Positive)
1.8	.70	.60	.40
1.9	.70	.66	.34
2.0	.57	.72	.28
2.1	.57	.78	.22
2.2	.57	.80	.20
2.3	.52	.83	.18
2.4	.44	.84	.16
n=601	p=0.000002	Std error=0.0489	

INTERPRETATION OF CLINICAL RESULTS

Spontaneous preterm labor and delivery is a multi-factorial disease. There is no single pathway leading to spontaneous preterm labor and delivery. Two pathways have been elucidated—a hormonal pathway and an infectious pathway. Salivary Estriol (SalEst™) is a marker for the hormonal pathway.

Spontaneous preterm labor and delivery is a low incidence disease, meaning the vast majority of women will deliver at term. A challenge with traditional risk scoring is accurately identifying those women who will deliver at term.

The strong negative predictive value (NPV) of the SalEst™ test (98%) means that it is highly unlikely that preterm labor and delivery will occur in women with low SalEst™ levels. The high specificity (92%) means that SalEst™ correctly identifies more than 92% of women who will deliver at term.

Conversely, traditional risk scoring misses 50-80% of those women who will ultimately deliver preterm. A sensitivity of 42-64% means that the SalEst™ test identifies approximately half of the spontaneous preterm labor and delivery patients, including those patients unidentified by traditional risk scoring. The positive predictive value (PPV) of 18.5% means that nearly 1 in 5 women identified by SalEst™ as high risk will experience spontaneous preterm labor and delivery. Having a positive SalEst™ test carries a relative risk (R.R.) of 7.8, which means these women are nearly 8 times as likely to have spontaneous preterm labor and delivery as those with low estriol levels. Preterm birth is a prior pregnancy, one of the most predictive of traditional risk factors, carries a relative risk (R.R.) of 2.6.

In summary, the clinical benefit of using SalEst™ as a screen for spontaneous preterm labor and delivery is that it identifies the vast majority of women as low risk (over 90%), and these women are very likely to deliver at term. For those women identified at risk with a positive rescreen, their relative risk is 7.8 in the combined population, which results in a 3-fold enhancement over the most predictive traditional risk assessment method. For example, a prior preterm birth, one of the most predictive of traditional risk factors, only carries a relative risk of 2.6.

REFERENCES

1 Jackson, G.M., McGregor, J.A., Lachelin, G.C.L., Goodwin, T.M., Artal, R. and Dullien, V., Salivary estriol rise predicts preterm labor. AM. J. Obstet. Gynecol., 1995, SPO Abstract.532.

2 Lachelin, G.C.L., McGarrigle, H.H.G., McGregor, J.A., Jackson, G.M., Goodwin, T.M., Artal, R. and Dullien, V., Premature rise in saliva estriol in women going into preterm labor. Abstract British Congress of Obstetrics and Gynecology. Dublin, Ireland, 1995.

3 McGregor JA; Jackson GM; Lachelin GC; Goodwin TM; Artal R; Hastings C; Dullien V, Salivary estriol as risk assessment for preterm labor: a prospective trial, Am J Obstet Gynecol Oct; 1995, 173(4): 1337-42.

4 Centers for Disease Control. Low birthweight - United States. Morb Mortal Wkly Rep 1990; 39:137-52.

5 Main PJ, Ernest JM, Moore ML. Causes of low birthweight in public and private patients. Am J Obstet Gynecol, 1987; 1135-1168.

6 Main, DM, Gabba SG, Richard D, Srong S. Can preterm deliveries be prevented? Am J Obstet Gynecol, 1985;151:892-1168.

7 Danforth's Obstetrics and Gynecology. Sixth Edition, Scott, JR.; J.B. Lippincott Company, Philadelphia, 1990:70-71.

8 ACOG Technical Bulletin, Preterm Labor. Number 133, October 1989

9 Biswas, A., Chaudhury, A., Chatboraj, S.C. and Dale, S.L., Do catechol estrogens participate in the initiation of labor. Am J Obstet Gynecol 1991 Oct;165(4 Pt 1):984-7.

10 Anderson, J.H., Pack, E.F. and Clark, J.H., Estrogen-induced uterine responses and growth: Relationship to receptor estrogen binding by uterine nuclei. Endocrinolology, 1975. 96(1): 169-7.

11 Klopper, A., Masson, G., et al., Estriol in Plasma, A compartmental study. Amer. J. Obstet. Gynecol., 1973 117:21-26

12 Young, B.K, Jirku, H., Kadnes, S. and Levitz, M., Renal clearances of estriol conjugates in normal human pregnancy at term. Am. J. Obstet. Gynecol., 1976;126:38-42.

13 McGarrigle, H.H.G. and Lachelin, G.C.L., Unconjugated and conjugated oestrogens in the late pregnancy saliva. In Second Joint Meeting of British Endocrine Societies, 5 -8 April (1983a).

14 Fischer-Rasmussen, W., Gabrielsen, M.V. and Wisborg, T., Relation of estriol in saliva to serum estriol during normal pregnancy. Acta. Obstet. Gynecol. Scand., 1981, 60:417-420

15 Lachelin, G.C.L. and McGarrigle, H.H.G., A Comparison of saliva, plasma unconjugated and plasma total oestriol levels throughout normal pregnancy Br. J. Obstet. Gynecol., 1984, 91: 1203-1209

16 Tulchinsky, D. and Abraham, G.E., Radioimmunoassay of plasma estriol. J. Clin. Endo. Metab., 1971, 33: 775-782

17 Darne, J., McGarrigle, H.H.G. and Lachelin, G.C.L., Saliva oestriol, oestradiol, oestrone and progesterone levels in pregnancy: spontaneous labor at term is preceded by a rise in the saliva-oestriol:progesterone ratio Br. J. Obstet. Gynaecol., 1987, 94: 227-235

18 Darne, J., McGarrigle, H.H.G. and Lachelin, G.C.L., Increased saliva oestriol to progesterone ratio before spontaneous preterm labor delivery: a possible predictor for preterm labor? Br. Med. J., 1987, 294: 270-272

19 Mercer, B.M., Goldenberg, R.L., Das, A., et.al., The preterm prediction study, a clinical risk assessment system Am. J. Obstet. Gynecol., 1998, 174: 1885-1995

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YOUR DOCTOR HAS PRESCRIBED THE SALEST™ SYSTEM TO PROVIDE IMPORTANT INFORMATION ABOUT YOUR PREGNANCY.

When labor and birth take place before the 37th week of pregnancy, the result is a ***preterm birth.**** Many women who experience ***preterm birth*** may not have specific health problems or past medical conditions to alert doctors that they are at risk.

The SalEst System is available to help doctors identify the risk of ***preterm labor**** and birth. With the SalEst System, sample collection can be done in the privacy of your home. It is an easy and reliable way to help your doctor provide the care you and your baby need.

*See Words To Know Section on page 18

CONTENTS

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INTRODUCTION TO THE SALEST SYSTEM

Your doctor has prescribed the SalEst System. It is a safe, convenient way to help your physician identify your risk for ***preterm birth**** and birth. This series of tests is an important part of your prenatal care. When there is risk of ***preterm birth***, doctors can often take steps to improve your care.

You may have heard about various kinds of home tests. An example is the home pregnancy test. The SalEst System is different because it is directed by your doctor and the testing is done at a laboratory. However, the sample for testing can be collected by patients at home or in their doctor's office. This is more convenient than making a special trip to a laboratory to provide a sample for testing. But collecting a sample at

*See Words To Know Section on page 18

home means you will have an active role in the testing. It is important that you follow the instructions carefully so the test results will be reliable. If you have questions about any of the steps for collecting a test sample, please call your doctor, nurse or our toll-free number: **1-888-404-BIEX.**

The steps for collecting a test sample for the SalEst System are not difficult. You will provide a sample of saliva. This will be tested at our laboratory to determine the level of a hormone, ***estriol.**** From the very beginning hormones play an important role in pregnancy. Research found that ***estriol*** increases gradually during the first two ***trimesters**** of pregnancy. ***Estriol*** increases more rapidly in the last ***trimester*** when it helps start the labor process. An increase in this hormone after 22 weeks of pregnancy and before 36 weeks can be a signal that labor may soon begin.

Testing for *estriol* every one to two weeks from 22 weeks to 36 weeks of pregnancy gives your doctor more information to determine the kind of care that is best for you and your baby.

You will need to follow some basic steps for the test to be most reliable. Ship the sample to the laboratory on the day you collect the sample. Carefully follow each step in the Directions for Use that are in your SalEst Sample Collection Kit.

Your doctor will use the results of the SalEst System along with other information to determine whether you are at risk of having a ***preterm**** baby. If you are at risk, this test will help your doctor make a plan of care for you and your baby.

*See Words To Know Section on page 18

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The SolBet System provides important information about your pregnancy. Your doctor can use the SolBet System along with other clinical information to determine if you are at risk for preterm labor and birth.

17. 7

Collecting and testing ***estriol**** in saliva is a valuable step to determine your risk for ***preterm labor**** and delivery. If the result of your SalEst test is **positive,** it does ***not*** mean you are certain to have a ***preterm birth****. It may mean that the normal process of labor may have started early. A positive test allows you and your doctor to consider the risk of preterm labor and birth and work to reduce that risk. You may be asked to send in another saliva sample immediately after a test result that is positive.

If the result of your SalEst test is **negative,** it does **not** mean you are certain **not** to have a ***preterm birth.*** It is, however, a sign that preterm labor is very unlikely to occur in the next two to three weeks. **It is still important that you report any sign or symptom of preterm labor to your doctor and follow his or her instructions carefully.**

Because you will collect your saliva without a doctor or nurse present, it is important that you follow directions carefully. **And it is important that you ship the sample to the laboratory for testing the same day that you collect the sample.**

If any of the steps for saliva collection is overlooked – or if you forget to ship the sample in time – the results of the test will not be as reliable. Ask your doctor or nurse if you have any questions or call our toll-free number: **1-888-404-BIEX.**

It is important to mark your calendar to indicate each date your doctor wants you to collect a saliva sample.

*See Words To Know Section on page 18

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GENERAL QUESTIONS ABOUT PRETERM BIRTH

What is preterm birth?

A term pregnancy ends with delivery after 37 weeks. Preterm or premature birth is delivery that occurs before 37 weeks.

Why is preterm birth a concern?

Babies born preterm have not had the full amount of time they need to develop. If babies are born very early, they may have very low birthweight. These preterm babies may have serious health problems. They may require special care and they may need to spend more time in the hospital in the NICU ***(Neonatal Intensive Care Unit*)***. This special care often overcomes the problems of preterm birth.

*See Words To Know Section on page 18

The NICU provides babies with the extra nourishment, oxygen and warmth they need. This special care is often successful, but it is far better to keep a baby in the mother's ***womb****** for as long as it is safe and good for the baby.

Who is at risk for preterm birth?

Until recently, doctors found it difficult to predict if a woman was likely to deliver preterm.

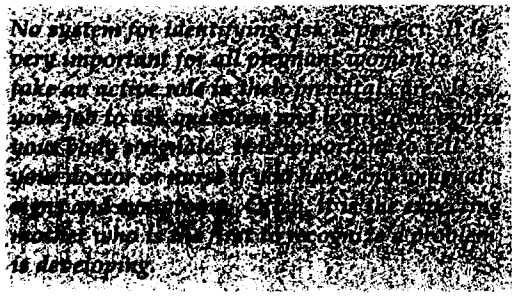
Only about half of the women who have preterm birth can be identified by "***risk screening****." A doctor screens a woman for risk by looking at her medical history, such as preterm birth in a previous pregnancy. A doctor also looks at behaviors, such as smoking. And a doctor looks at a woman's lifestyle, such as a physically demanding job, unusual stress, and environmental factors. This ***risk screening*** is usually done early in pregnancy and it is repeated in the third ***trimester****. This method of ***risk screening*** helps find problems that may lead to preterm birth early in the pregnancy. Identification of preterm labor is important because many problems can be treated.

It is important to know that *risk screening* by itself is not always successful in identifying many women who are at risk. Sometimes women who seem very healthy and have no signs of risk can develop preterm labor. In fact, more than half of the women who experience preterm birth are not identified through traditional ***risk screening***.

*See Words To Know Section on page 18

It is also important to know that many women who appear to be at risk based on the *risk screening** process never develop problems. These women may be given extra care that isn't needed. They may experience a lot of stress and worry.

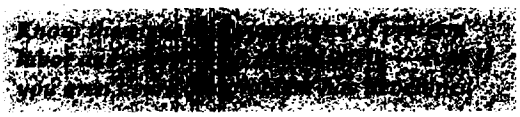
The SalEst System provides doctors with a valuable tool to predict risk of preterm birth. It is based on increased production of the hormone, *estriol**. The system provides additional information that helps a doctor decide if a patient needs extra care and treatment. This system is used to assess the risk of preterm labor as early as 22 weeks of pregnancy.



*See Words To Know Section on page 18

YOUR ROLE IN REDUCING THE RISK OF PRETERM BIRTH

- Give your doctor complete information about your health, family medical history, lifestyle and behaviors.
- Follow your doctor's prenatal care instructions.
- Keep all your prenatal care appointments.
- Follow your doctor's program for good nutrition and exercise.



WARNING SIGNS OF PRETERM LABOR

- **Vaginal Discharge**
  An increase in the amount or a change in type (watery, mucous or bloody)
- **Pressure in the pelvic area or abdomen**
- **Low, dull backache**
- **Abdominal cramps, with or without diarrhea**
- **Regular contractions (even if they are very faint) or uterine tightening**
- **Flu like symptoms**

WORDS TO KNOW

In this section we will provide definitions for new technical terms that are used to describe your care and the care of your baby. If you do not understand a term, please feel free to ask your physician or care provider what these terms mean.

Estriol A hormone that acts as a communication link between the mother and unborn baby. High estriol levels have been shown to be associated with the start of labor and are produced by the baby and the mother.

Neonatal Having to do with the newborn (up to 1 month old).

Neonatal Intensive Care Unit A baby born preterm, usually weighing less than normal birthweight, may be admitted to this special care unit in the hospital. Preterm babies may require oxygen, extra warmth, feeding tubes, and many other treatments because of health problems caused by being born too soon.

Preterm Birth (Preterm Delivery) When a pregnancy ends before 37 weeks it is considered PRETERM. A TERM pregnancy ends after 37 weeks.

Preterm Labor Contractions of the uterus along with cervical changes before the 37th week of pregnancy which can result in preterm birth.

Risk Screening Identification of risk for problems in a woman's pregnancy.

Trimester One of the three month periods of pregnancy. Pregnancy is divided into three trimesters.

Womb or uterus The organ that surrounds and protects the baby during pregnancy.


reassurance


reassurance

For more information
about the SalEst System,
call 1-888-404-BIEX

Biex

Manufactured by Biex, Inc.
6693 Sierra Lane, Suite F, Dublin, CA 94568

Sal
SYSTEM

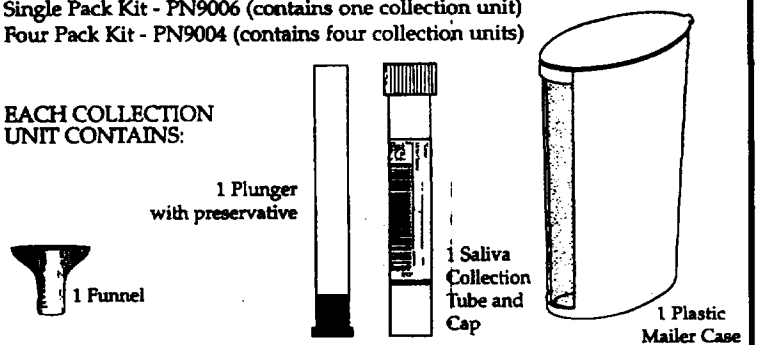
Saliva Collection Kit

The Biex SalEst Saliva Collection Kit is a safe, easy and reliable way for your doctor to identify the risk of preterm labor and birth. Under your doctor's direction, you collect saliva samples at the doctor's office or at home, then send them to a laboratory for testing. Your doctor will tell you how often to take a sample. Make sure to report any problems to your doctor. It is important that you follow the directions carefully so the test results will be accurate.

For In Vitro Diagnostic Use

CONTENTS:
Single Pack Kit - PN9006 (contains one collection unit)
Four Pack Kit - PN9004 (contains four collection units)

EACH COLLECTION UNIT CONTAINS:



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Store at Room Temperature

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WAIT

1 hour after eating and drinking

1 hour after smoking

1 hour after chewing gum

1 hour after toothbrushing, flossing or using mouthwash

WHEN YOU SHOULD COLLECT YOUR SALIVA

(saliva collection can take up to 10 minutes)

- **COLLECT** saliva between 9 am and 8 pm only.
- **REMEMBER** writing the time on the label is important.
- **WAIT** 1 hour after eating, drinking, smoking, chewing gum, toothbrushing, flossing or using mouthwash.

- **RINSE** your mouth with water and wait 10 minutes.

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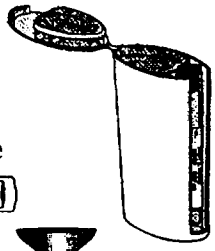
HOW TO COLLECT YOUR SALIVA

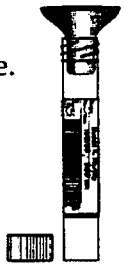
1. **REMOVE** tube and plunger from the plastic case.



PRINT your name, the date and time on the tube label.

2. **REMOVE** cap and funnel from case. Remove plunger from tube.

3. **PLACE** funnel in end of tube.

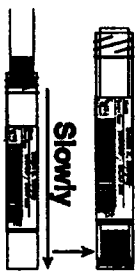


4. **REST** funnel against your lower lip. Let your saliva flow into the tube up to the **GREEN** line on the label.

Note: Do not allow mucus or phlegm from lower throat to enter the tube.

Saliva - not foam - should reach the green line.

5. **PLACE** the green rubber end of the plunger into the tube and slowly push down all the way to the bottom.



If it is hard to push, place your fingers on the rim of the plunger while pushing down.

How to Prepare and Mail Your Sample

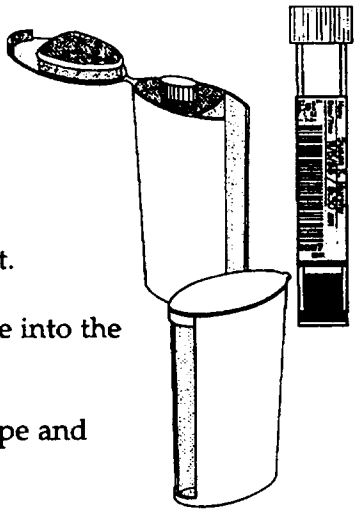
6 **PLACE** the cap on the tube and screw it on tightly.

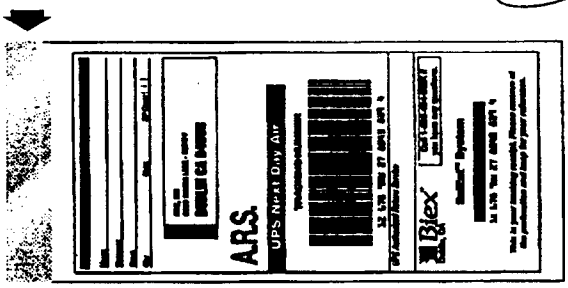
7 **PLACE** tube in center of plastic case.

SNAP top of plastic case shut.

8 **PLACE** the sealed plastic case into the self-addressed envelope.

9 **PULL** blue tab to seal envelope and send by UPS promptly.





DO NOT PULL BLUE TAB UNTIL AFTER PLASTIC CASE IS INSERTED INTO THE ENVELOPE.

If you have any questions, please contact your doctor, nurse or Biex.

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Clinical Monograph

SALEST™ Test

Salivary Estriol:
A risk assessment marker
for spontaneous preterm labor
and delivery

Intended Use

The SalEst® test is intended to detect and measure by enzyme-linked immunosorbent assay (ELISA) technology the level of salivary estriol in pregnant women. The device is indicated for use as an aid in identifying risk of spontaneous preterm labor and delivery in singleton pregnancies. The device can be used every 1 to 2 weeks from gestational ages 22 to 34 weeks. The test should be used as a component of the clinician's assessment of risk for preterm labor and delivery.

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The Enigma Of Preterm Birth

Preterm birth is the leading cause of neonatal morbidity and mortality. Preterm labor is a multifactorial disorder that arises from a diversity of maternal and fetal complications.[1] Two pathways have been elucidated to precipitate spontaneous preterm labor: a hormonal pathway and an infectious pathway.[2,3-5] Salivary estriol is a marker for the hormonal pathway.

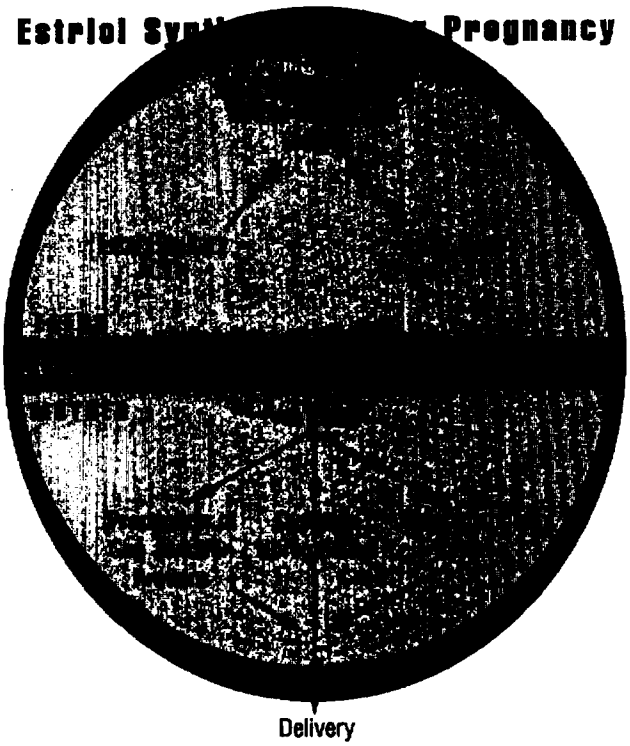
Ideally, etiology-specific prevention of preterm labor represents the most expedient approach to reducing its incidence. However, most current risk assessment methods are not etiology-specific and fail to identify half of the women destined to develop preterm labor. Unfortunately, their ability to assess risk in the primigravida is even less accurate. At the present time, risk for preterm labor is often assessed on the basis of a patient's medical history, lifestyle, behavior, and demographic profile.[6] The most commonly used risk scoring systems are based on the original Papiernik system, which was later modified by Creasy.[6-8]

There is a compelling need for new tools that can identify pregnancies at risk for preterm labor that are etiology-specific. As a new biochemical marker for the hormonal pathway that leads to preterm labor, salivary estriol can aid pregnancy management by objectively identifying the risk.

Salivary Estriol And Preterm Birth

Estriol, a member of the estrogen family, plays a significant role in pregnancy and the parturition process.[2,9,10] A rise in estriol, which can be monitored in the saliva, has been shown to precede delivery at term, as well as prior to spontaneous preterm birth.[11,12]

Over the past two decades, saliva has become an increasingly attractive matrix for monitoring therapeutic drugs, certain diseases, and hormone levels.[13] Saliva levels reflect the concentrations of free, unconjugated steroid hormones in the plasma. The advantages of saliva as a matrix are its accessibility, noninvasive collection procedure, and stability, all of which contribute to patient compliance and satisfaction.



In a normal pregnancy, estriol first appears at week 9 of gestation, and its plasma concentration increases throughout gestation, along with estrone and estradiol.[2,10,14] Approximately five weeks before term, concentrations of estriol begin rising more steeply than the other two estrogens, and continue their ascent until term.[15] Estriol is the most abundant estrogen in late pregnancy.[2]

Estriol performs many pregnancy-related functions. One of its primary roles is as a biochemical communicator from the fetus to the mother. An elaborate biochemical modification pathway assures that estriol production is coordinately controlled by fetus, placenta, and mother.[10] The process of estriol production is initiated by the release of adrenocorticotropic hormone (ACTH) from the fetal pituitary, which triggers the release of estriol precursors (DHEAS) from the fetal adrenal gland. The fetal liver subsequently converts DHEAS by 16-hydroxylation into

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the immediate precursor to estriol, 16-α-hydroxy-dehydroepiandrosterone (16-α-OH-DHEAS). 16-α-OH-DHEAS is then converted to estriol by the placenta.[10,14,16] Important roles for estrogens in coordinating parturition are likely to include increasing the release of oxytocin from the maternal hypothalamus, augmenting the number of oxytocin receptors, enhancing the production of myometrial gap junction proteins, increasing the release of decidual prostaglandins via the oxytocin receptors and decidua, ripening the cervix, and augmenting uterine blood flow.[2,14,17]

Feasibility Trial

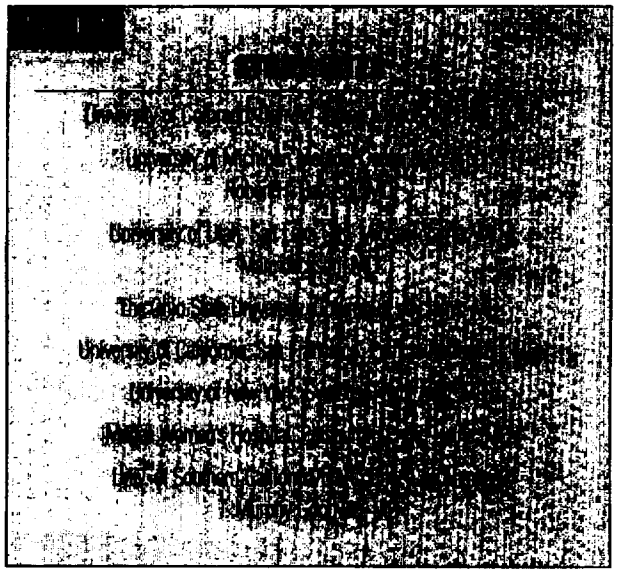
A trial was conducted at five medical centers in the U.S. and the U.K. to determine the feasibility of using salivary estriol as an indicator of risk for preterm labor.[12] The expression of salivary estriol, which was determined weekly in 190 women, exhibited a similar pattern in women with term and preterm births. However, the estriol surge began sooner in women who developed preterm labor such that equivalent values were attained approximately four weeks earlier. A concentration of 2.1 ng/ml salivary estriol or above was predictive of risk based upon receiver/operator curve (ROC) characteristics.

Clinical Success With The Salivary Estriol Test

A pivotal trial was conducted to examine whether salivary estriol ≥2.1 ng/ml can be used prospectively as a risk assessment marker for spontaneous preterm labor.[18]

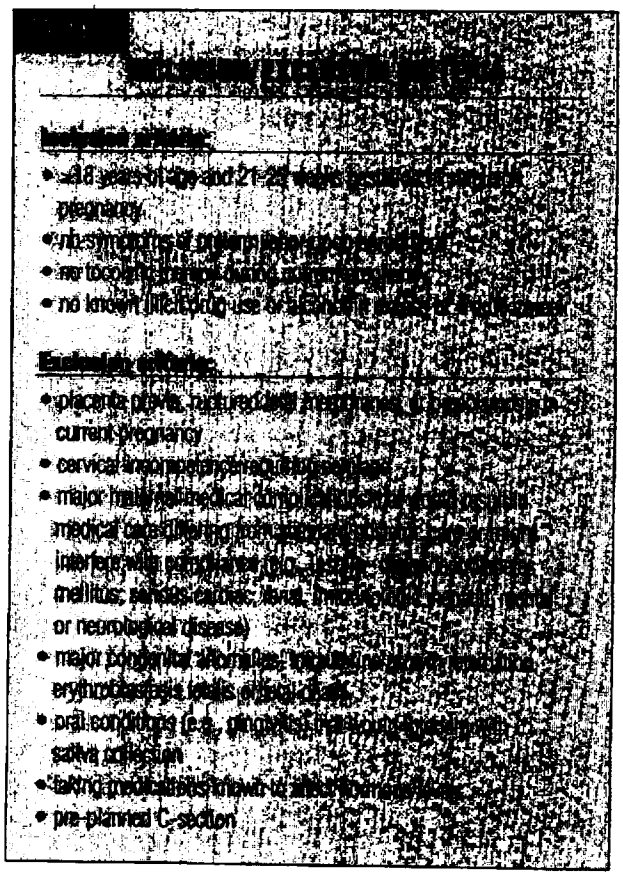
Study Design

- Title: *"Monitoring of Salivary Estriol as a Risk Assessment Marker for Idiopathic Preterm Labor in High and Low Risk Women"*
- A prospective, longitudinal, triple-blinded study was conducted at eight U.S. sites (see Table 1).
- Saliva samples were collected weekly, alternately at the home and the clinic, beginning at week 21-25 until delivery. Specimens were sent in mailers



to the clinic after home collection (see Table 2).

- A salivary estriol cutoff of ≥2.1 ng/ml designated a positive test prior to 36 weeks of gestation. Salivary estriol levels were analyzed by ELISA.
- Clinic visits were scheduled at baseline and at biweekly intervals. The baseline examination



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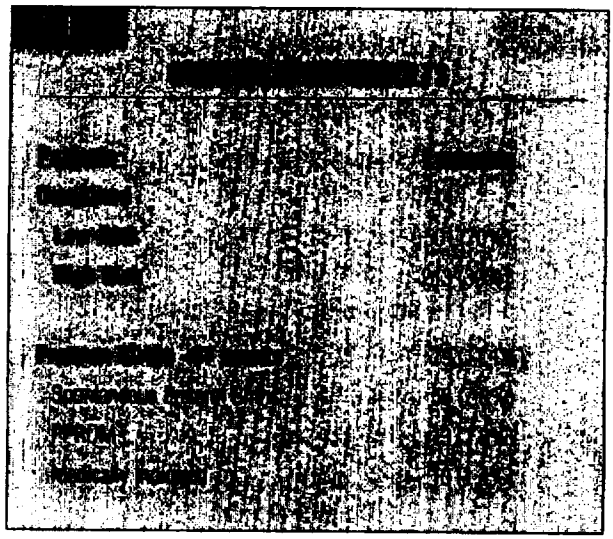
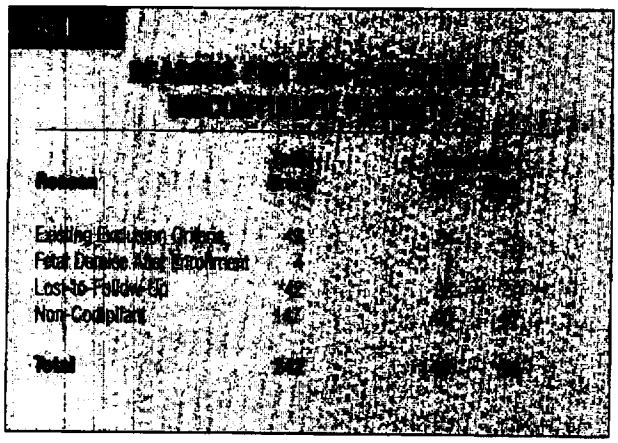


included checking for signs and symptoms of preterm labor, rupture of membranes, or leakage of amniotic fluid, and performing a digital vaginal examination to check the cervix. Subsequent clinic visits included questioning the patient for symptoms of preterm labor; cervical examination every four weeks beginning at week 27; and saliva collection.

- Saliva was collected between 9 am and 8 pm, since no significant variation in estriol levels has been found between these hours.[19] No food, drink, smoking, tooth brushing, mouth washing, or gum chewing was permitted during the hour prior to collection. The patient was instructed to rinse her mouth with water, wait 10 minutes, and then collect 1 ml of saliva into the tube provided.
- Preterm labor was defined as spontaneous preterm labor with intact membranes which resulted in a preterm delivery within 72 hours of onset, prior to 37 weeks of gestation.
- Risk status was determined by the Creasy scoring method, such that high-risk patients had a Creasy score of ≥10 and low-risk patients had a score of <10.
- Gestational age was determined by LMP, or by ultrasound if patient was unsure of LMP. If there was greater than a 14-day difference between the two, ultrasound was taken to be the more definitive.

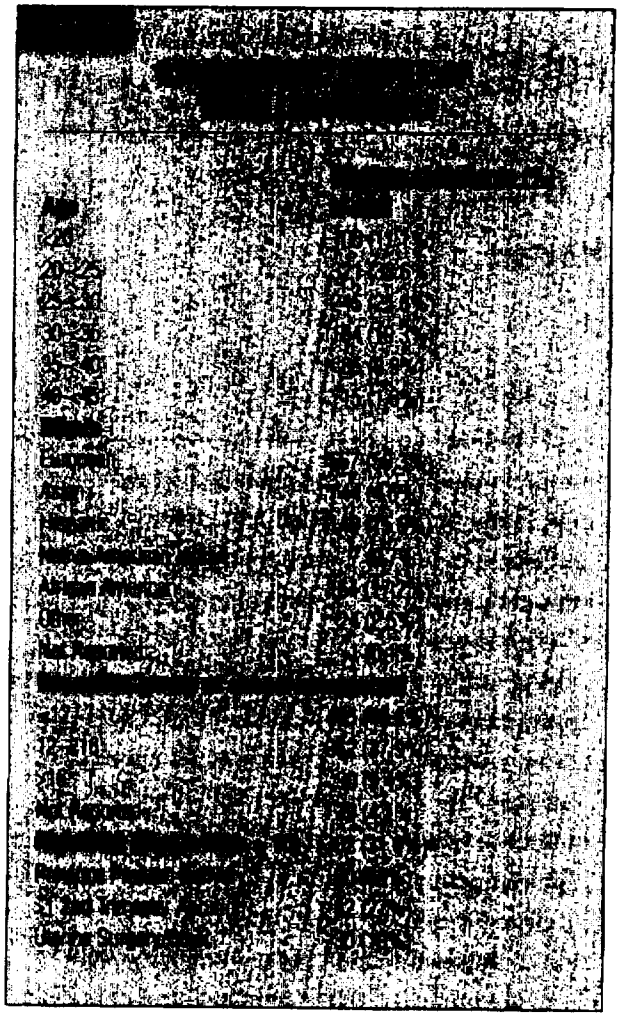
Clinical Endpoints

- **Primary clinical endpoint:** To compare the incidence of preterm labor and delivery among women with salivary estriol levels ≥2.1 ng/ml to women with levels <2.1 ng/ml prior to 36 weeks of gestation (Fishers exact test, $p \leq 0.05$).
- **Secondary clinical endpoint:** To compare the predictive accuracy of salivary estriol monitoring with the modified Creasy method (McNemar test, $p \leq 0.05$).



Demographic Results

- Of the 956 enrolled patients, 714 were evaluable: 213 high-risk and 501 low-risk (see Table 3).
- The primary reasons for nonevaluability or discontinuation from the study were patient noncompliance and the presence of exclusion criteria (see Table 4).
- The primary analysis was done on two groups (n=601): PTL/PTD without betamethasone or tocolytics and term deliveries without preterm labor, betamethasone or tocolytics. Subjects removed from the primary analysis included pPROMS, medically indicated preterm births, patients treated with betamethasone and/or tocolytics and term patients with preterm labor.
- Overall, the study population was diverse with respect to age, ethnicity, and education (see Table 5).
- The demographics of women who achieved 2.1 ng/ml salivary estriol prior to labor and delivery were similar to the demographics of the study



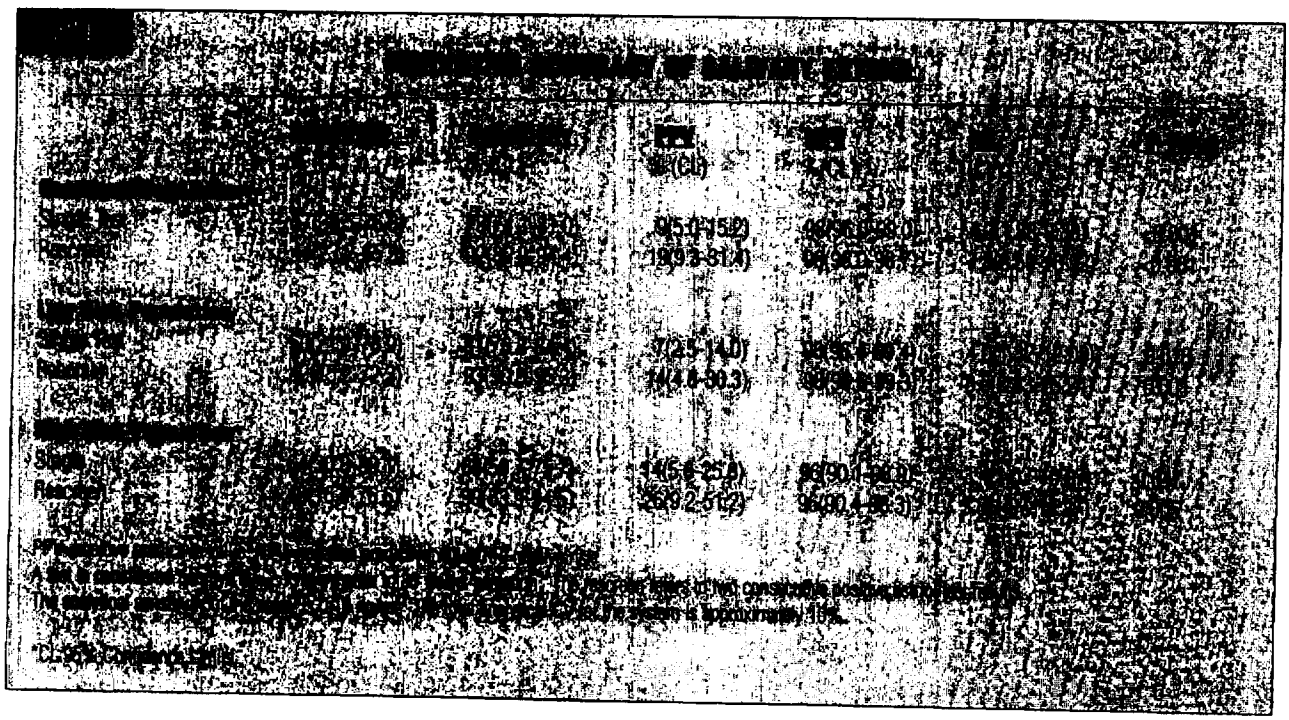
population as a whole. Estrogen metabolism does not appear to be different among subpopulations of women.

Efficacy Results

- The clinical performance characteristics of the SalEst test are shown in Table 6 for both a single test and a rescreen test. The rescreen refers to a second consecutive positive test result, collected after an initial positive screen. Use of the rescreen test helps reduce false positives.

Statistical significance of the primary and secondary endpoints was achieved

- A sensitivity of 42-57% indicates that the SalEst test identified approximately half of the spontaneous preterm labor and delivery patients.
- The positive predictive value (PPV) of 19% on the SalEst rescreen in the combined population indicates that nearly 1 in 5 women identified by SalEst as high risk will develop spontaneous preterm labor and delivery. The positive predictive value in the high risk population was 26% with the rescreen test. The rescreen reduces false positives.



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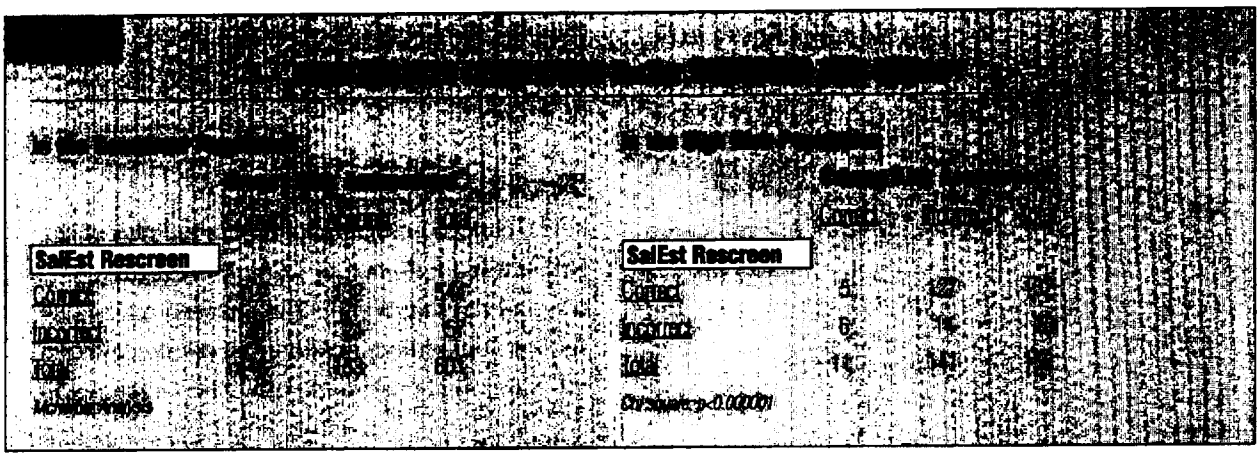


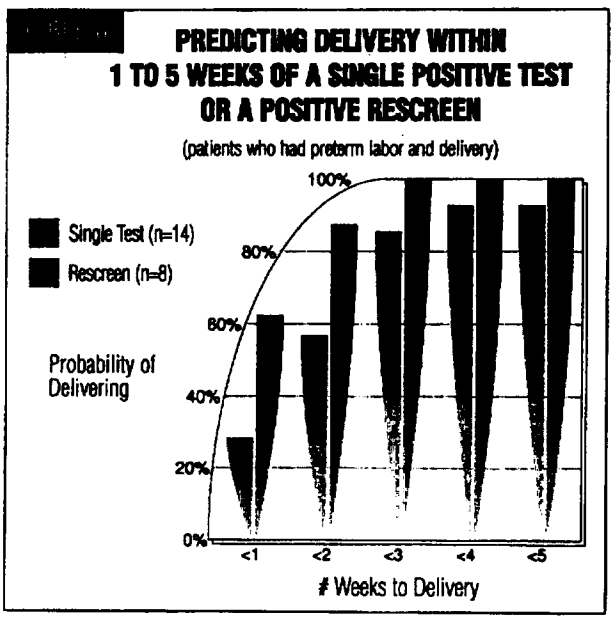
• A positive SalEst test carries a relative risk (RR) of 7.8 in the combined population, which indicates that women who have a positive rescreen test result are 7.8 times more likely to develop spontaneous preterm labor and delivery.

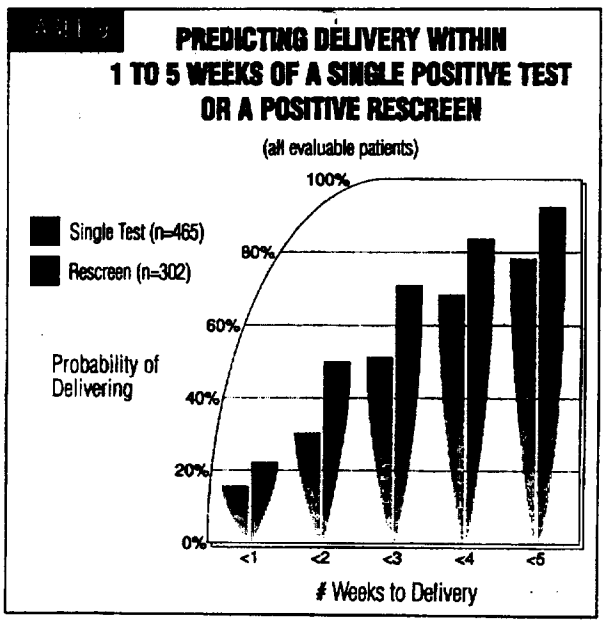
• A significant component of the clinical utility of the SalEst test is its negative predictive value (NPV). The strong NPV of 98% in the combined population indicates that it is highly unlikely that preterm labor and delivery will occur in women with low SalEst levels. The high specificity (92%) in the combined population indicates that SalEst correctly identifies 92% of women who will deliver at term.

• Compared to traditional risk factors, salivary estriol testing significantly improved the ability to predict preterm labor and delivery. In the low-risk population SalEst identified 50% of the cases of spontaneous preterm labor and delivery that were missed by traditional risk assessment methods.

• When the SalEst test is compared to Creasy scoring, salivary estriol correctly predicts the appropriate outcome 91% of the time, while the Creasy method correctly predicts the appropriate outcome 75% of the time (see Table 7). This difference was even more pronounced in the high-risk group, in which salivary estriol correctly predicted the appropriate outcome 87% of the time, compared to the Creasy method, which correctly predicted the appropriate outcome 7.2% of the time (McNemar test, $p<0.001$).

• For those women identified as being at risk, the



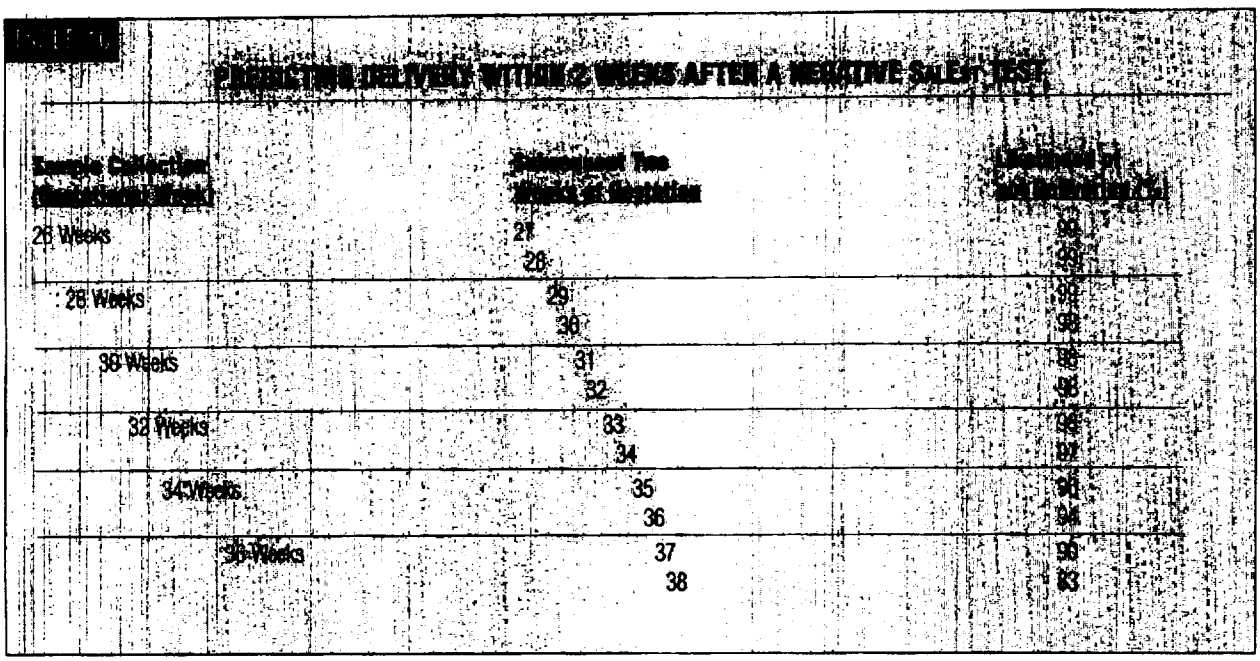


degree of risk is significantly greater than that identified by traditional risk factors. A positive rescreen, which carries an RR of 7.8 in the combined population, results in a 3-fold enhancement over the most predictive traditional risk assessment methods. For example, a prior preterm birth, one of the most predictive of traditional risk factors, only carries an RR of 2.6.[20]

- A single positive test and rescreen demonstrated the clinical utility of SalEst test in predicting the probability of delivering within a five-week time frame, regardless of gestational age.
- Among women who had preterm labor and delivery and who had a positive SalEst test, the rescreen enhanced the accuracy of predicting delivery within one to five weeks. In this subpopulation, women who had a positive rescreen had a 63% chance of delivering within one week, an 88% chance of delivering within two weeks, and a 100% chance of delivering within three weeks (see Table 8).
- In order to explore whether what occurred in the subpopulation that developed preterm labor and delivery reflected the underlying biology of salivary estriol, the time to delivery of the entire study population that had at least one positive SalEst test result (n=465) was examined. Again, the rescreen enhanced the accuracy of predicting delivery within one to five weeks.
- A single positive SalEst test indicates a 78% likelihood of delivering within five weeks, compared to a 92% likelihood of delivering within five weeks after a positive rescreen (see Table 9). Similarly, a positive rescreen indicates a 71% likelihood of delivering within three weeks, and an 84% likelihood of delivering within four weeks. The time to delivery from elevated estriol is one to three weeks in the majority of women.

Clinical Utility

- The SalEst test should be used as a component of the clinician's assessment of risk for preterm labor and delivery.
- In the event of a single positive SalEst test result, further monitoring for other risk factors of preterm birth, including a salivary estriol rescreen, would be indicated. The patient could be contacted and reassessed for current risk factors of preterm labor, receive a physical examination, and be educated in the signs and symptoms of preterm labor. If the SalEst rescreen test result is also positive, high-risk care should be maintained. No further SalEst testing is necessary.
- A negative test result predicts the likelihood of



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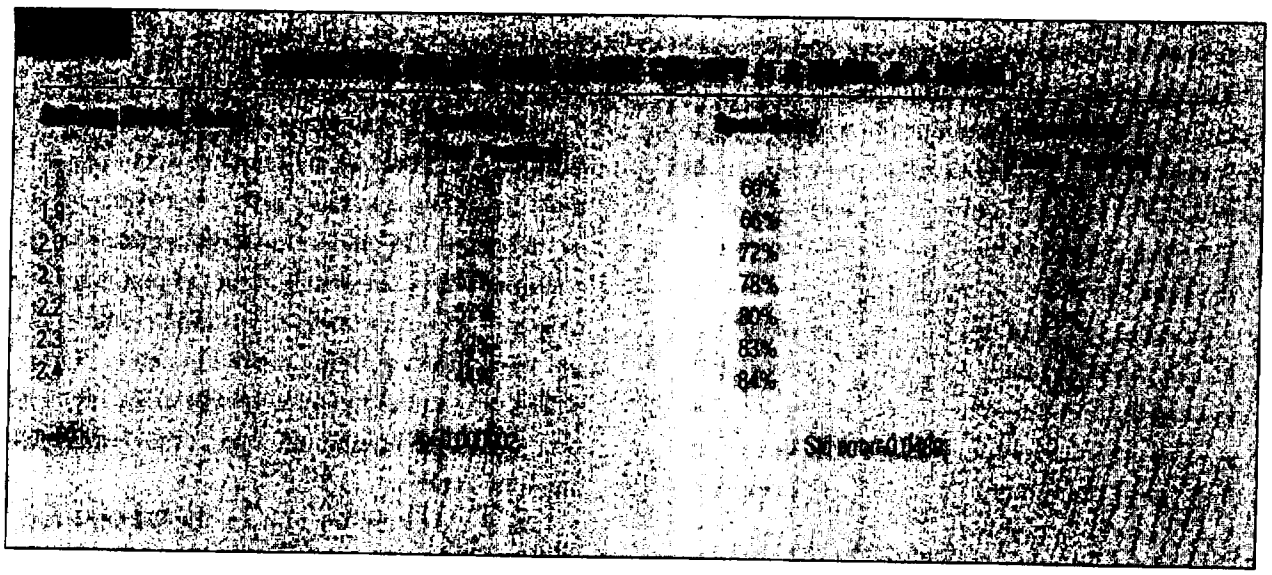
not delivering within the ensuing two weeks (see Table 10). For example, when a sample collected at 26 weeks is negative, the woman has a 99% chance of not delivering within the next week, and a 99% chance of not delivering before week 28. When she collects her next sample, at week 28 if samples are collected biweekly, a new negative sample will project her chances of not delivering before week 30.

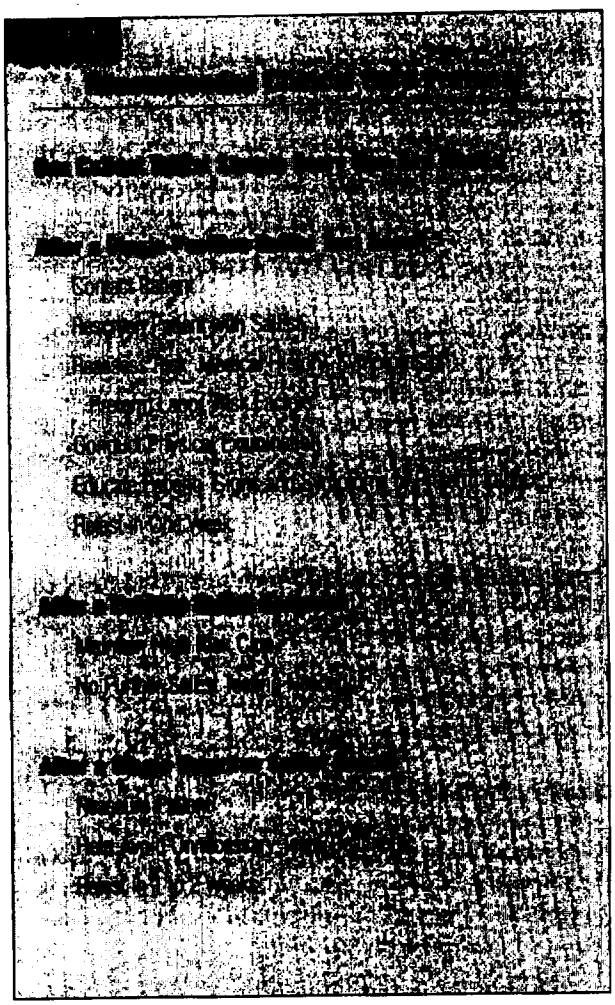
• Two clinical benefits of the high negative predictive value are minimization of unnecessary intervention and the reduction of anxiety.

• It is recommended that a salivary estriol value of 2.1 ng/ml or greater be considered a positive result; there may be clinical significance attached to estriol values above or below the 2.1 ng/ml cut-off. Cut-off values below 2.1 ng/ml may identify more true positives (increased sensitivity), but also result in more false positives. Conversely, cut-off values above 2.1 ng/ml may identify fewer true positives (decreased sensitivity), although they result in fewer false positives. A range of values around the 2.1 ng/ml cut-off, from ROC curves, is as follows (see Table 11):



Summary

- The SalEst test can be used as an aid in identifying risk of preterm labor and delivery.
- The risk status of women who are identified as being at-risk for preterm labor by SalEst is significantly higher than that identified by traditional risk factors.
- Salivary estriol is a biochemical risk assessment marker for preterm labor and delivery, based on an endocrine mechanism. The marker can be used prospectively to monitor women with singleton pregnancies.
- Based on a positive or negative Salivary Estriol test, physicians can change patient management, resulting in appropriate levels of care and use of resources for appropriate patients (see Table 12).
- The SalEst test is a screen for spontaneous preterm labor and delivery which identifies the vast majority of women as low risk (91%), and these women are very likely to deliver at term.
- The positive predictive accuracy of salivary estriol ≥2.1 ng/ml is greatest after two consecutive positive SalEst test results, collected a week apart.



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Appendix

Appendix 1
DEFINITIONS

Sensitivity measures the ability of the SalEst test to detect women who will have spontaneous preterm labor/delivery. The sensitivity is expressed as the percentage of women detected as positive by the test out of the total number of women who experienced spontaneous preterm labor/delivery. *Sensitivity = [TP/(TP+FN)]*

Specificity measures the ability of the SalEst test to detect those women who will not have spontaneous preterm labor/delivery. The specificity is expressed as the percentage of women detected as negative by the test out of the total number of women who did not experience spontaneous preterm labor/delivery.
Specificity = [TN/(FP+TN)]

Positive Predictive Value (PPV) is the ability of the SalEst test to predict those women who will have spontaneous preterm labor/delivery. PPV is expressed as the percentage of women who will have spontaneous preterm labor/delivery out of the total number of women measured as positive by the test. *PPV = [TP/(TP+FP)]*

Negative Predictive Value (NPV) is the ability of the SalEst test to predict those women who will not have spontaneous preterm labor/delivery. NPV is expressed as the percentage of women who will not have spontaneous preterm labor/delivery out of the total number of women measured as negative by the test.
NPV = [TN/(TN+FN)]

Relative Risk (RR) is a measure of the likelihood of spontaneous preterm labor/delivery occurring for a woman with a positive SalEst test result as compared to a woman with a negative result. The value is calculated from the ratio of PPV and (1-NPV), i.e., *RR = PPV/(1-NPV)*

P-Value is a measure of the likelihood that chance alone could have accounted for an observed difference. The smaller the p-value, the less likely it is that chance alone could explain the observed results. By convention, a p-value must be equal to or less than 0.05 to be considered significant. If, for example, the p-value associated with an RR of 3 is less than 0.05, it means that less than 5 times in 100 would such a large RR be observed based on chance alone. Therefore, it is most likely that the RR of 3 was due to the effectiveness of the test.

Appendix 2
COLLECTION OF SALIVA

Instructions for the patient are provided with the Biex Saliva Collection Kit.

Saliva is collected weekly or biweekly from week 22 through week 36 of gestation.

The Collection Kit is composed of individual self-contained shrink-wrapped collection units. Each collection unit contains a capped saliva collection tube, a plunger filter with preservatives, and a funnel to aid in collecting the saliva. These components are contained within a molded plastic case that serves to provide leakproof mailing of sample collections. Contained within the mailer is an absorbent pad to collect any leakage. Patient instructions and a pre-addressed mailer to return the sample to the laboratory are packaged with the plastic case in a shrink-wrapped box.

The collection tube consists of a 13 mm X 100 mm test tube with a screw-cap closure. The tube has a label that includes an indicator band that serves to provide visual confirmation that sufficient volume, 1 ml, has been provided. The label also contains a bar code that identifies the tube. The collection tube also consists of a filter plunger device that slides into the test tube after the sample has been provided. The plunger serves the dual purpose of filtering and introducing anti-microbial agents into the sample. After sealing the collection tube with the plunger in place, the filter creates an inner tube sealed on one end by the filter and on the opposite end by the screw cap. In that way the sample can be utilized at the assay lab and sampled from the inner tube without the potential difficulties of particulate interference with the pipetting process.

The collection units are packaged in either of two configurations. Four units will be packaged in a 4-pack Collection Kit. The 4-pack Collection Kit contains four collection units, a patient insert, and calendar reminders. Each 4-pack Collection Kit will be assigned a unique kit identification number at the time of assembly. The identification number will be associated with the bar code identification number on each tube within the collection units in the kit. This database of kit ID and tube ID numbers will be used to provide positive tracking of patient samples.

The collection units will also be packaged as single Collection Kits. The single Collection Kit will contain one collection unit, a patient insert and an enrollment card. The enrollment card will be used by the physician to enroll the women into the screening program, as well as to provide identification and medical history information for the laboratory. The single Collection Kit enrollment form will also include a tracking identification number that has been linked to the collection tube within its respective single-pack kit in a database at the time of assembly.
NOTE: Saliva should be collected between 9 a.m. and 8 p.m.

Appendix 3
REPORTING AND INTERPRETATION OF TEST RESULTS

- Patient specimens with estriol values equal to or greater than 2.1 ng/ml are positive.
- Test results should be interpreted in conjunction with the patient's clinical presentation and other diagnostic test results. A negative result by any method does not rule out the possibility of preterm labor and delivery.
- The physician should interpret positive results with caution. The physician should encourage the patient to submit an additional sample to confirm the initial positive result.

Appendix 4
LIMITATIONS

The Biex SalEst™ test is used as an aid in identifying pregnant women with singleton pregnancies at risk for spontaneous preterm labor and preterm delivery. The test should not be used alone in pregnant women with suspected rupture of membranes, vaginal infections, uterine anomalies or those who are otherwise already diagnosed as having preterm labor and/or when treatment with betamethasone and tocolytics is being administered. The SalEst test should not be used in conjunction with bleeding gums, intrauterine growth retardation, or the presence of fetal demise. The test should be used as a component of the clinician's assessment of risk for preterm labor and delivery.

Salivary estriol is designed as a screening tool to identify pregnancies at risk for preterm labor, rather than as a diagnostic tool. Risk assessment with salivary estriol is compromised by prior treatment with betamethasone. To avoid the confounding effects of betamethasone, salivary estriol should be used as a component of the clinician's assessment of risk for preterm labor and delivery.

Specimens should be measured either within two hours of collection or after twenty four (24) hours. Specimens measured 3-4 hours after collection could have artificially lower values.

REFERENCES

1. American College of Obstetricians and Gynecologists. Preterm Labor. Washington, DC: *ACOG Technical Bulletin*, No. 133. October, 1989.
2. Fuchs A-R, Fuchs F, Stubblefield PG. *Preterm Birth*. New York: McGraw Hill. 1993.
3. Carroll SG, Sebire NJ, Nicolaides KH. *Preterm Prelabour Amniorrhexis*. Pearl River, New York: Parthenon Publishing Group. 1996.
4. McGregor JA, French JI, Parker R, et al. Prevention of premature birth by screening and treatment for common genital tract infections: results of a prospective controlled evaluation. *Am J Obstet Gynecol*. 1995;173:157-187.
5. Romero R, Mazor M. Infection and preterm labor. *Clin Obstet Gynecol*. 1988;31:553-584.
6. American College of Obstetricians and Gynecologists. Preterm Labor. Washington, DC: *ACOG Technical Bulletin*, No. 206. June, 1995.
7. Papiernik E. Coefficient de risque d'accouchement premature. *Presse Med*. 1969;77:793-794.
8. Creasy RK, Gummer BA, Liggins GC. System for predicting spontaneous preterm birth. *Am J Obstet Gynecol*. 1980;55:692-695.
9. Anderson JN, Peck EJ, Clark JH. Estrogen-induced uterine responses and growth: relationship to receptor estrogen binding by uterine nuclei. *Endocrinology*. 1975;96:160-167.
10. Speroff L, Glass RH, Kase NG. The endocrinology of pregnancy. In: *Clinical Gynecologic Endocrinology and Infertility*, 4th ed. New York: Williams & Wilkins. 1994.
11. Goodwin TM, Jackson GM, McGregor JA, et al. Increased incidence of preterm labor and preterm delivery associated with increased salivary estriol level. *Am J Obstet Gynecol*. SPO Abstracts. January, 1996. Abstract 59.
12. McGregor JA, Jackson GM, Lachelin GCL, et al. Salivary estriol as risk assessment for preterm labor: a prospective trial. *Am J Obstet Gynecol*. 1995;173:1337-1342.
13. Malamud D, Tabak L, eds. Saliva as a Diagnostic Fluid. *Annals of the New York Academy of Sciences*. 1993;694.
14. Gabbe SG, Niebyl JR, Simpson JL, ed. *Obstetrics*, 3rd ed. New York: Churchill Livingstone. 1996.
15. Darne J, McGarrigle HHG, Lachelin GCL. Saliva oestriol, oestradiol, oestrone and progesterone levels in pregnancy: spontaneous labour at term is preceded by a rise in the saliva oestriol: progesterone ratio. *Br J Obstet Gynecol*. 1987;94:227-235.
16. Lavery JP, ed. *The Human Placenta*. Rockville: Aspen Publishers. 1987.
17. Hedriana HL, Parry S, Gilbert WM. Clinical correlation of salivary estriol concentration and cervical ripening: a possible role for fetal estriol in the onset of parturition. *Am J Obstet Gynecol*. SPO Abstracts. January, 1996. Abstract 631.
18. Salivary Estriol Premarketing Approval Application. Clinical Section, 6.3. July, 1997.
19. McGregor J, Barrett J, Hastings C. Diurnal variation of salivary estriol in pregnancy. *Am J Obstet Gynecol*. SPO Abstracts. January, 1997. Abstract 167.
20. Mercer BM, Goldenberg RL, Das A, et al. The preterm prediction study: a clinical risk assessment system. *Am J Obstet Gynecol*. 1996;174:1885-1895.

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